Correlation between blood and milk serum leptin in goats and growth of their offspring

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ABSTRACT: Boer and Boer crossbred meat-type does were used in two experiments to determine whether goat milk serum contains leptin and to investigate possible correlations of milk and serum leptin in does and subsequent growth of their offspring. Blood and milk samples were collected within 2 h of kidding (d 0) from 20 (Exp. 1; spring) or 22 does (Exp. 2; the following fall). Blood milk samples were then collected again on d 0.5, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 (Exp. 1) or d 0.5, 1, 2, 3, 4, 5, 6, 7, 14, and 21 (Exp. 2). Body weights of kids were recorded on d 0, and BW of kids and does were recorded weekly beginning on d 7 (kids) or 21 (does), with BCS also recorded for does beginning on d 28 for Exp. 1 and on d 0.5, 1, 2, 3, 4, 5, 6, 7, 14, and 21 for Exp. 2. Leptin was detected in colostral milk and was influenced by days postpartum, decreasing ($P < 0.001$) over time with an average of 4.4 ± 0.3 ng/mL (Exp. 1) and 18.1 ± 1.0 ng/mL (Exp. 2) on d 0 compared with 1.0 ± 0.3 ng/mL on d 56 (Exp. 1) and 2.9 ± 0.2 ng/mL on d 21 (Exp. 2). Day postpartum and milk serum leptin were negatively correlated ($P < 0.001$) for Exp. 1 (r = –0.27) and Exp. 2 (r = –0.46). For Exp. 1 only, blood serum leptin tended ($P = 0.09$) to be influenced by day, with a weak positive correlation (r = 0.15; $P < 0.02$). Weak positive correlations ($P < 0.01$) were found between blood serum leptin and doe BCS (r = 0.42 in Exp. 1, and r = 0.13 in Exp. 2) and doe BW (r = 0.44 in Exp. 1, and r = 0.26 in Exp. 2), with the absence of a stronger relationship likely due in part to the short time period measured and the lack of significant changes in BCS and BW during that time. In conclusion, leptin was present in milk and blood serum of does, and blood serum leptin was weakly correlated with doe BW and BCS, but it was not related to kid BW. Therefore, further studies are needed to clarify the relationships involving milk and serum leptin in goats.

Key Words: Correlation, Growth, Leptin, Meat Goats, Milk

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Introduction

Leptin is a 16-kDa protein secreted from adipocytes (Houseknecht et al., 1998) involved in several physiological functions, including regulation of food intake, energy expenditure, body temperature regulation, and whole body metabolic balance. Leptin is produced by fat cells, but it has also been found in the placenta (Hassink et al., 1997; Hoggard et al., 1997; Ashworth et al., 2000), where it passes through to the fetus during its transition to the neonate (Matsuda et al., 1999). In addition, leptin has been found in the milk of humans (Houseknecht et al., 1997), as well as in some livestock species (Estienne et al., 2000; McFadin et al., 2002; Salimei et al., 2002). Therefore, it is likely that leptin also is found in goat milk and that it may be related to the BW and/or body condition of the female. However, the possible effects of milk leptin on the growth of goat kids are unknown, and little research has been conducted involving leptin in goats. Therefore, the objectives of our two experiments were to determine whether goat milk serum contains leptin and to investigate possible relationships among milk and serum leptin, BW, and BCS in does, and subsequent growth (BW) of their offspring.

Materials and Methods

Animals and Procedures

In both experiments, mixed-parity Boer and Boer crossbred meat-type does, approximately 2.8 ± 0.2 yr of age, were placed in a 2.5-ha drylot area at least 4 d before the first expected kidding date. Does were al-

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lowed ad libitum access to mixed grass hay (approximately 10% CP, DM basis) and water and fed approximately 0.45 kg/doe daily of a corn–soybean meal diet (mixed to be approximately 14% CP, as-fed basis) with trace minerals added. After kidding, does and their kids were placed in individual 1.5 × 1.5 m pens in a barn with open ends and windows to allow for a natural light:dark cycle and to maintain temperatures above freezing. Average mean local temperatures ranged from 0 to 28°C for Exp. 1 and from 0 to 18°C for Exp. 2 (based on National Oceanic and Atmospheric Administration historical data). Pens housing the goats were on concrete floors with straw bedding, and animals remained in pens for 56 (Exp. 1) or 21 d (Exp. 2). Animals were allowed ad libitum access to water and were presented with grass hay (same hay as above) and the corn–soybean meal feed described above twice daily at levels meant to maintain at least a 2.5 BCS (on a scale of 1 to 5, with 1 being very thin and 5 being obese; adapted from Russel, 1991). All animal-related procedures were conducted in compliance with University of Maryland Eastern Shore Institutional Animal Care and Use Committee guidelines.

Twenty does and their offspring (1.9 ± 0.15 kids per doe) were used in Exp. 1 in the spring. Blood and milk samples were collected within 2 h of kidding (d 0) and again on d 0.5, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56. Body weights of kids were recorded at d 0, and BW and BCS (does only) of kids and does were subsequently recorded weekly beginning on d 7 (kid BW), d 21 (doe BW), or d 28 (doe BCS) and continuing until d 56. For Exp. 2, 22 does and their offspring (2.3 ± 0.17 kids/doe) were used in the fall. Based on results from Exp. 1, more intensive sampling was conducted in the early postpartum period and the duration of sampling was shortened to 21 d (most changes in leptin were noted by that day in Exp. 1). Doe blood and milk samples and kid and doe BW were collected within 2 h of kidding (d 0) and again on d 0.5, 1, 2, 3, 4, 5, 6, 7, 14, and 21. Blood was stored at 4°C and allowed to clot overnight. Serum was then collected after centrifugation at 1,200 × *g* for 20 min and stored at –20°C. Milk samples were ultracentrifuged at 100,000 × g at 5°C for 1 h, and the clear supernatant fraction (milk serum) was extracted and stored at –20°C. Blood serum and milk serum leptin concentrations were measured using the leptin RIA described by Delavaud et al. (2000) and previously validated for use with milk serum (McFadin et al., 2002).

Statistical Analyses

Data from the two experiments were analyzed separately due to differences in sampling protocol and season of the year. Pearson product moment correlations between the variables doe milk serum leptin, doe blood serum leptin, doe BW, doe BCS, days postpartum, and kid BW were calculated using the CORR procedure of SAS (SAS Inst., Inc., Cary, NC). This procedure produces single bivariate correlation coefficients and related significance tests, automatically generating the Pearson r (Dilorio and Hardy, 1996). Partial correlations also were obtained using day and/or number of nursing kids as the controlling variable when applicable (for kid BW and doe milk and blood serum leptin). A partial correlation measures the strength of a relationship between two variables, while controlling the effect of one or more additional variables. The MIXED procedure of SAS was used to calculate the effect of day on milk and serum leptin concentrations. The GLM procedure of SAS also was used with the MANOVA option to obtain possible effects of day as well as partial correlation coefficients, with results similar to those obtained with MIXED and CORR procedures with day as the controlling variable.

Results

Experiment 1

Milk leptin was influenced by day postpartum, such that as days postpartum increased, milk serum leptin decreased ($P < 0.001$; Figure 1). By d 3, milk leptin reached a nadir and remained unchanged throughout the rest of the sampling period (Figure 1), perhaps resulting in the weak but significant negative correlation noted between days postpartum and milk serum leptin ($r = –0.27$; $P < 0.001$). In contrast, doe blood serum leptin tended to be influenced by day, increasing ($P = 0.09$) with days postpartum, such that leptin concentrations at d 14, 28, 35, 43, and 49 were greater ($P < 0.03$) than d-0 leptin concentrations, presumably resulting in the weak positive correlation between days postpartum and serum leptin noted ($r = 0.15$; $P < 0.02$; Figure 1). Partial correlation coefficients using day and number of suckling kids as controlling variables indicated positive correlations between serum blood leptin and doe BCS ($r = 0.32$; $P < 0.002$) and doe BW ($r = 0.46$; $P < 0.001$). Without using controlling variables, initial correlations for serum blood leptin were $r = 0.42$ ($P < 0.001$) with doe BCS and $r = 0.44$ ($P < 0.001$) with doe BW. There were no relationships noted between milk serum leptin and kid BW ($P = 0.56$) or milk serum leptin and blood serum leptin ($P = 0.16$).

Experiment 2

Days postpartum influenced concentrations of milk serum leptin such that leptin decreased ($P < 0.001$) as days postpartum increased, with concentrations reaching a nadir on d 4 and remaining at that level for the rest of the sampling period (Figure 2). A negative correlation existed between days postpartum and milk serum leptin ($r = –0.46$; $P < 0.001$), whereas no relationship was found between blood serum leptin and days postpartum (Figure 2). Accounting for days postpartum and number of suckling kids, doe blood serum leptin was weakly and positively correlated to doe BCS and doe BW ($P < 0.001$; $r = 0.18$ and $r = 0.25$, respectively). Without

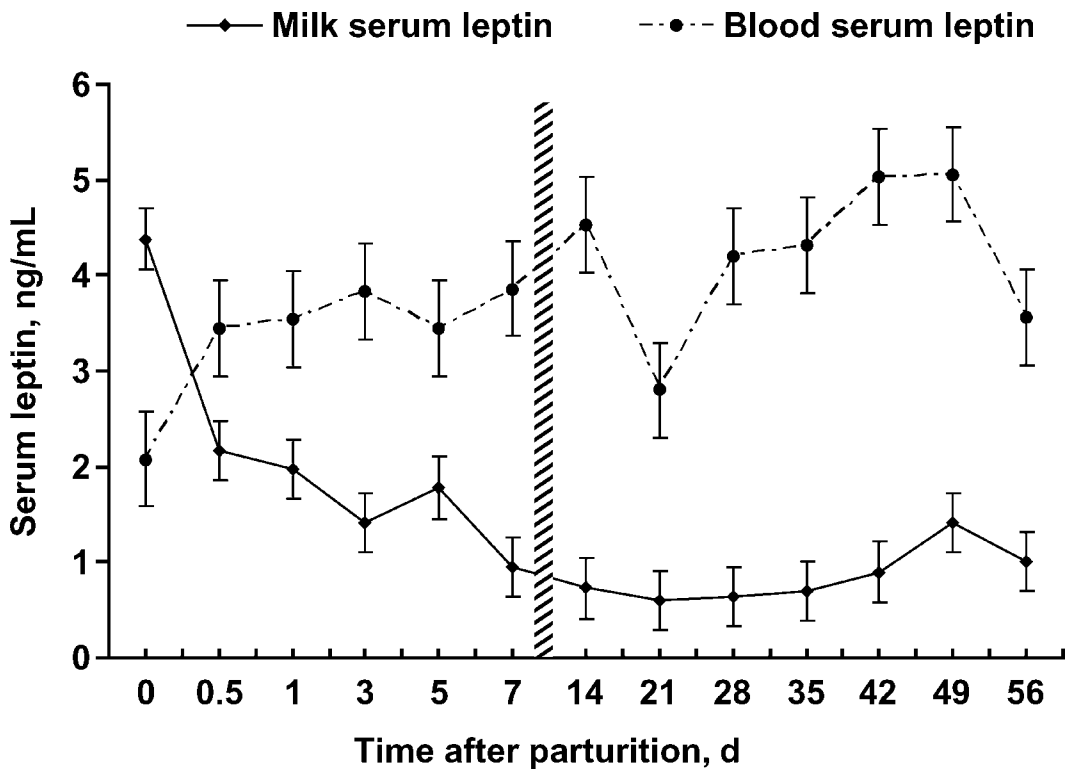

Figure 1. Milk and blood serum leptin concentrations (mean ± SEM) in 20 meat-type does kidding in the spring (Exp. 1) from samples collected within 2 h after parturition (d 0) to 56 d postpartum. The gray bar denotes a change in sampling schedule. As days postpartum increased, milk serum leptin decreased ($P < 0.001$), whereas doe blood serum leptin tended ($P = 0.09$) to increase with days postpartum.

controlling for day postpartum or number of suckling kids, the correlations with blood serum leptin were r = 0.13 with doe BCS ($P < 0.07$) and r = 0.26 with doe BW ($P < 0.001$). There were no relationships noted between milk serum leptin and kid BW ($P = 0.25$) or milk serum leptin and blood serum leptin ($P = 0.37$).

Discussion

Leptin is found in the milk of humans and mice (Houseknecht et al., 1997; Aoki et al., 1999), as well as in livestock species such as pigs, sheep, and horses (Estienne et al., 2000; McFadin et al., 2002; Salimei et al., 2002). Leptin mRNA also has been found in the mammary tissue of humans, rats, and sheep (Smith-Kirwin et al., 1998; Aoki et al., 1999; Laud et al., 1999), and it is regulated in bovine mammary epithelial cells by growth factors known to alter mammary function and nutrient partitioning (Smith and Sheffield, 2002).

In the present study, leptin was found in the milk serum of mixed-parity Boer crossbred meat-type does. As days postpartum increased in both studies, the concentrations of leptin in doe milk serum decreased, with the greatest milk serum leptin concentrations occurring in colostral milk samples. These results are similar to those reported in sheep (McFadin et al., 2002), mares (Salimei et al., 2002), and pigs (Estienne et al., 2000), and this effect has been attributed to a pooling of leptin in the udder before parturition (McFadin et al., 2002). The general protein profile in milk follows the same pattern (Fuertes et al., 1998) as that of milk leptin, being greatest in colostral or early lactation samples and decreasing over time, indicating that leptin is probably not differentially modulated. Therefore, the peak in milk leptin occurs when neonates are best able to absorb large proteins through the gastrointestinal tract. For example, elevated blood serum leptin has been found in neonatal suckled vs. unsuckled rat pups (Dessolin et al., 1997), in rat pups fed milk plus leptin vs. milk alone (Casabiell et al., 1997), and in neonatal pigs treated orally with leptin (Whitley et al., 2001) or suckled vs. being fed milk replacer (Weiler et al., 2002). Increased serum leptin concentrations also were found in breast-fed compared with formula-fed human infants (Savino et al., 2004). Therefore, leptin certainly has the potential to play a role in the development of the neonate, although specific effects are still not understood.

Initial theories of possible milk leptin influence on the neonate were through feed intake and/or potential growth regulation, but those theories have not been fully supported by research. Although a negative relationship was found between early weight gain (up to 1 mo of age) and breast milk leptin (measured on d 15 postpartum) in infants (Dundar et al., 2005), and 4-d-old rat pups treated chronically with five times the

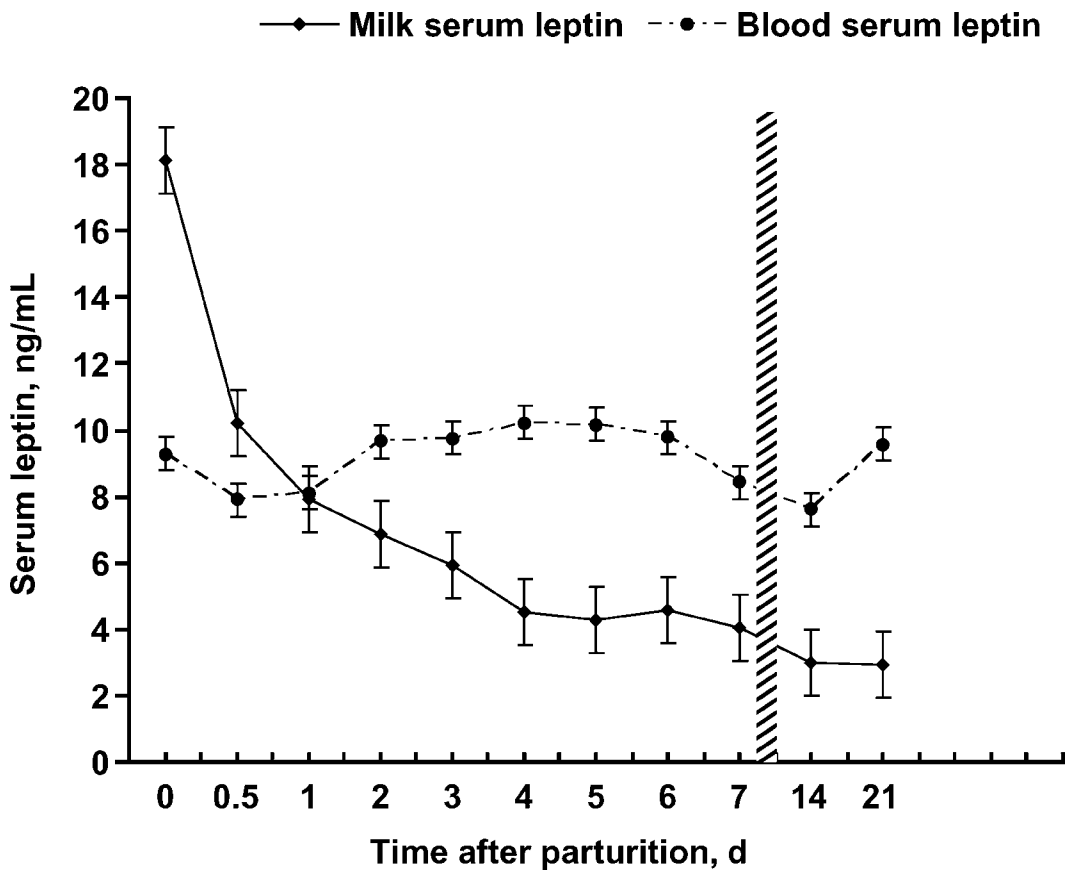

Figure 2. Milk and blood serum leptin concentrations (mean ± SEM) in 22 meat-type does kidding in the fall (Exp. 2) for samples collected within 2 h after parturition (d 0) to 21 d postpartum. The gray bar denotes a change in sampling schedule. As days postpartum increased, concentrations of milk serum leptin decreased ($P < 0.001$), whereas no relationship was found between blood serum leptin and days postpartum ($P = 0.84$).

expected ingestion concentrations of leptin had less gastric contents than controls (Sanchez et al., 2005), exogenous leptin did not influence feed intake by neonatal mice 7 to 10 d of age (Mistry et al., 1999). Moreover, leptin in breast milk did not influence satiation at the end of suckling in human infants (Ucar et al., 2000); however, delayed effects of early leptin influence could be possible. In piglets suckled or fed with a milk substitute plus injected daily with either dexamethasone or a placebo from d 5 through 20, piglet plasma leptin was predicative of bone and fat mass after accounting for body size and treatment (Weiler et al., 2002). Intake was not measured in the current study, however, and no apparent relationships between dam milk leptin and offspring growth were noted. Therefore, the present study upholds the current lack of convincing evidence for leptin as a direct modulator of neonatal growth; however, milk leptin could be important for indirect developmental effects, kid survival, or other factors not measured in this study. For example, in neonatal pigs, physiological concentrations of leptin added to artificial milk replacer normalized maturation of the small intestinal mucosa to the range found in those nursing the sow (Wolinski et al., 2003), indicating a possible role for leptin in modulation of neonatal gut development.

Neonatal thermoregulation also may be affected by ingestion of milk leptin. Leptin was shown to alter sympathetically mediated themoregulatory thermogenesis to augment cold defense abilities in rat pups (Stehling et al., 1997). In addition, in neonatal rats, the ability of leptin to accelerate metabolic rates was acquired early in life (by d 17), and leptin was theorized to promote survival of neonates (Mistry et al., 1999). In neonatal lambs, Mostyn et al. (2002) reported that leptin administration prevented the normal decrease in colonic temperature over the first few hours and days after birth. In addition, at 7 d of age, colonic temperature was strongly correlated with uncoupling protein 1 mRNA abundance and thermogenic potential in leptin-treated lambs (Mostyn et al., 2002). Uncoupling proteins are characteristic of brown adipose tissue, which is important for thermogenesis in neonates of many mammalian species. Like lambs and rats, neonatal goats also possess brown adipose fat at birth, which markedly decreases over the first few weeks of life (Trayhurn et al., 1993). Coincidentally, in the present study, although not compared statistically, blood serum leptin and colostral milk serum leptin concentrations were numerically greater for does kidding in the fall, in cooler weather, than in those kidding in the spring,

although BCS were similar, indicating a possible influence of season of the year or seasonal temperature. An effect of season on blood serum leptin concentrations has been noted recently for horses (Buff et al., 2005), but further research specifically designed to determine possible effects of season on milk leptin in goats is needed.

Doe blood serum leptin was only weakly related to BCS and BW in the present study; this result was likely due in part to the lack of significant changes in BCS (average = 2.65 ± 0.02 for Exp. 1 and 2.77 ± 0.01 for Exp. 2) and BW (53.5 ± 0.82 kg for Exp. 1, and 55.4 ± 0.61 kg for Exp. 2) during the sampling periods and/or the relatively short sampling period (21 d for Exp. 1). In other species, there are reports of stronger positive relationships between circulating concentrations of leptin and body fatness (Maffei et al., 1995; Delavaud et al., 2000; Buff et al., 2001) and BW (Considine, 1996). Nonetheless, the weak relationship we noted between serum leptin and BCS is similar to results reported in ewes that maintained their BCS throughout lactation (McFadin et al., 2002; BCS = 2.86 ± 0.59).

In conclusion, leptin is present in the milk of goats and, as has been observed in other species, concentrations are greatest just after parturition and decrease quickly thereafter. Serum leptin was only weakly correlated with BCS and BW in does, and a link between milk leptin and kid growth was not realized. Therefore, further studies are needed to clarify the relationships involving milk and serum leptin in goats, including serum leptin relationships to BCS and/or BW and possible alternative effects of milk leptin on the neonate.

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